JACC Vol. 29, No. 7
June 1997:1447–53

A Prospective, Randomized, Double-Blind Multicenter Trial of a Single Bolus Injection of the Novel Modified t-PA E6010 in the Treatment of Acute Myocardial Infarction: Comparison With Native t-PA

CHUICHI KAWAI, MD, FACC, YOSHIKI YUI, MD,* SAICHI HOSODA, MD,†
MASAKIYO NOBUYOSHI, MD, FACC,‡ SHIN SUZUKI, MD,§ HIKARU SATO, MD,‖
FUMIMARO TAKATSU, MD,¶ TAKESHI MOTOMIYA, MD, FACC,#
KATSUO KANMATSUSE, MD, FACC,** KAZUHISA KODAMA, MD, FACC,††
YOSHIMASA YABE, MD, FACC,‡‡ TAKAZO MINAMINO, MD,§§ SHIN-ICHI KIMATA, MD,‖ ‖
MITSUYOSHI NAKASHIMA, MD, PHD,¶¶ ON BEHALF OF THE E6010 STUDY GROUP##

Kyoto, Tokyo, Kita-Kyushu, Hiroshima, Aichi, Osaka and Hamamatsu, Japan

Objectives. **This prospective, randomized, double-blind multicenter trial evaluated the efficacy and safety of a single bolus injection of the novel modified tissue-type plasminogen activator (t-PA) E6010 in the treatment of acute myocardial infarction compared with that of native t-PA.**

Background. **E6010 is a novel modified t-PA with a prolonged half-life ($t_{1/2}$ alpha ≥23 min) compared with native t-PA ($t_{1/2}$ alpha = 4 min). E6010 can be administered in patients as a single intravenous bolus injection, and early recanalization can be expected.**

Methods. **The efficacy of E6010 was compared with that of native t-PA in 199 patients with acute myocardial infarction who were treated within 6 h of onset in a prospective, randomized, double-blind multicenter trial. Patients were given either 0.22 mg/kg body weight of E6010 intravenously over 2 min or native t-PA (tisokinase) 28.8 mg or 14.4 million IU (10% of the total dose over 1 to 2 min, the remainder infused over 60 min).**

Results. **The primary end point was the recanalization rate of the infarct-related coronary artery at 60 min after the start of treatment. Time to reperfusion was shorter in the E6010 group than in the native t-PA group. Thrombolysis in Myocardial Infarction flow grade 2 or 3 recanalization at 15, 30, 45 and 60 min after administration was observed in 37%, 62%, 74% and 79% (95% confidence interval [CI] 70% to 87%) of the E6010-treated patients and in 14%, 32%, 50% and 65% (95% CI 55% to 74%) of native t-PA-treated patients, respectively (p = 0.032 at 60 min).**

Conclusions. **The present study indicates that, compared with native t-PA, a single bolus injection of E6010 over 2 min produces a higher rate of early recanalization of the infarct-related coronary artery without fatal bleeding complications.**

(J Am Coll Cardiol 1997;29:1447–53)

The aim of thrombolytic therapy for acute myocardial infarction is rapid lysis of thrombi in the infarct-related coronary artery to restore blood flow as early as possible. However, the widely used thrombolytic agent native tissue-type plasminogen activator (native t-PA) is administered by intravenous infusion because of its short half-life. The angiographic substudy of the Global Utilization of Streptokinase and TPA for Occluded Coronary Arteries (GUSTO) trials (1) indicated that front-loaded alteplase resulted in both a higher percentage of early vessel patency and significantly better survival than streptokinase. However, the front-loaded regimen followed by continuous intravenous infusion is cumbersome.

In the novel modified t-PA E6010, cysteine 84 in the epidermal growth factor domain has been replaced by serine, resulting in a plasma half-life ($t_{1/2}$ alpha ≥23 min) longer than that of native t-PA ($t_{1/2}$ alpha = 4 min) (2,3). Because of its longer half-life, E6010 can be administered intravenously as a single bolus injection. In a preliminary study (4), we reported that a single intravenous bolus injection of E6010 is effective in achieving early recanalization and a high recanalization rate,

From the Kyoto University, Kyoto; *Kyoto University Hospital, Kyoto; †Tokyo Women's Medical College, Tokyo; ‡Kokura Memorial Hospital, Kitakyushu; §Sakakibara Heart Institute, Tokyo; ‖Hiroshima City Hospital, Hiroshima; ¶Anjo Kosei Hospital, Anjo, Aichi; #Tokyo Metropolitan Hiroo General Hospital, Tokyo; **Nihon University Surugadai Hospital, Tokyo; ††Osaka Police Hospital, Osaka; ‡‡School of Medicine, Toho University, Tokyo; §§Sakurabashi Watanabe Hospital, Osaka; ‖ ‖Aoyama Hospital, Tokyo Women's Medical College, Tokyo; and ¶¶Hamamatsu University School of Medicine, Hamamatsu, Japan. ##A complete list of E6010 Study Group investigators and participating institutions appears in the Appendix. This study was presented in part at the Key Contribution Session of the 44th Annual Scientific Session of the American College of Cardiology, New Orleans, March 1995.

Manuscript received June 6, 1996; revised manuscript received February 13, 1997, accepted February 26, 1997.

Address for correspondence: Dr. Chuichi Kawai, Kyoto University, Nishiiru Karasuma Shimochojamachi-dori, Kyoto 602, Japan.

0735-1097/97/$17.00
PII S0735-1097(97)00074-0

Abbreviations and Acronyms

CI = confidence interval
GUSTO = Global Utilization of Streptokinase and TPA for Occluded Coronary Arteries
TIMI = Thrombolysis in Myocardial Infarction
t-PA = tissue-type plasminogen activator

and 0.22 mg/kg body weight of E6010 was established as the optimal dose. In the present study, we conducted a prospective, randomized, double-blind multicenter trial to examine the efficacy and safety of E6010 comparison with that of native t-PA in patients with acute myocardial infarction.

Methods

Patients. The patients provided informed consent, and the protocol was approved by the institutional review board of each of the 84 participating hospitals. Patients were enrolled between May 1993 and January 1994. The inclusion criteria were 1) treatment initiation within 6 h of onset of infarction; 2) total occlusion of the infarct-related coronary artery proved by coronary angiography after intracoronary administration of nitrates (isosorbide dinitrate or nitroglycerin); and 3) weight <100 kg and a negative skin test for allergy to the test drug. Age was not a criterion, although patients ≤75 years old were preferentially enrolled.

Patients were excluded if they satisfied any of the following criteria: 1) bleeding diathesis or hemorrhage symptoms (gastrointestinal or urinary tract bleeding, retroperitoneal or intracranial hemorrhage, hemoptysis, hemorrhagic diabetic retinopathy or other hemorrhagic eye diseases); 2) head injury, intracranial tumor, arteriovenous malformation or aneurysm; 3) history of cerebrovascular disease within the 6 months before the trial; 4) severe, poorly controlled hypertension (although no clear stipulations regarding blood pressure were made, the consensus among the clinicians participating in the study was that patients with systolic blood pressure >200 mm Hg and diastolic blood pressure >120 mm Hg should be excluded); 5) operation within 2 weeks of infarction; 6) severe complications, such as free ventricular wall rupture, ventricular septal perforation, papillary muscle rupture, multiple organ failure or cardiogenic shock unresponsive to vasopressor drugs; 7) prolonged cardiopulmonary resuscitation; 8) suspected left atrial thrombus (mitral stenosis associated with atrial fibrillation); 9) previous thrombolytic therapy for the infarction to be treated; 10) myocardial infarction as a complication of percutaneous transluminal coronary angioplasty; 11) history of hypersensitivity to vaccines or other biologic preparations; 12) serious liver or renal disease; and 13) pregnancy or suspected pregnancy.

Study design and medications. All patients underwent coronary angiography and administration of intracoronary nitroglycerin before receiving the fibrinolytic agent. Patients were then randomly assigned to receive either an intravenous bolus injection of 0.22 mg/kg of E6010 over 2 min, followed by infusion of native t-PA placebo dissolved in 100 ml of saline over 60 min (10% of the total native t-PA placebo dose was given as a bolus injection over 1 to 2 min; the remainder was infused over 60 minutes), or an intravenous bolus injection of E6010 placebo over 2 min, followed by infusion of 14.4 million IU (28.8 mg) of native t-PA dissolved in 100 ml of saline over 60 min (10% of the total dose given as a bolus injection over 1 to 2 min; the remainder was infused over 60 min). The native t-PA administration schedule conformed to the indications for use currently used in Japan.

E6010 (Eisai Co., Ltd., Tokyo, Japan) was supplied as a freeze-dried injectable preparation in vials containing 22 mg of the drug. The specific activity of E6010 is ~125,000 IU/mg, and it has a molecular weight of 68 kilodaltons. Native t-PA (tisokinase, Asahikasei Kogyo Co., Ltd., Tokyo, Japan) was also supplied as a freeze-dried preparation in vials for injection containing 3.6 million IU (7.2 mg) of the drug. A total of 5,000 U of heparin was given intravenously immediately before coronary angiography, during which intracoronary administration of a nitrate was used to demonstrate total occlusion of the infarct-related coronary artery, a patient eligibility criterion.

End points. The primary end point was Thrombolysis in Myocardial Infarction (TIMI) grade 2 or 3 recanalization of the infarct-related coronary artery at 60 min of treatment initiation; evaluation was performed according to the TIMI classification (5): *grade 0* = no reperfusion; *grade 1* = thrombolysis without myocardial reperfusion; *grade 2* = partial reperfusion; and *grade 3* = complete reperfusion. Secondary end points included recanalization of the infarct-related artery at 15, 30 and 45 min of treatment initiation and therapy-related bleeding complications. Only patients with an angiographically proved occlusion of the infarct-related artery were selected for the study.

Fibrinolysis assays. Blood samples were collected in citrate containing D-Phe-Pro-Arg-CH_2CI (PPACK) 1 mmol/liter (6). Blood samples were obtained just before and 60 and 120 min and 24 h after test drug administration and were then centrifuged. All plasma samples were stored at −20°C until analysis.

Plasminogen and alpha$_2$-plasmin inhibitor activity were assayed using the chromogenic substrate S-2251 test (7). Fibrinogen levels were measured using the clotting rate method (8); fibrinogen degradation product (D-dimer) levels were measured by enzyme-linked immunosorbent assay (9).

Statistical analysis and sample size estimation. All analyses were performed using the Statistical Analysis System (SAS Institute, Ltd., Tokyo, Japan) program and an NEC PC-9821 AP computer. Results are expressed as mean value ± SD. Group differences in baseline characteristics were assessed by the Student *t* and chi-square tests. Chi-square analysis was used to analyze differences in recanalization, reocclusion rates and bleeding complications. The subgroup effects of recanalization were investigated using the Cochran-Mantel-Haenszel test. A Wilcoxon signed rank test was used to assess changes in fibrinolytic variables versus pretreatment values. All tests were

two-tailed and were performed at a level of significance of $p < 0.05$.

The study was scheduled to be performed with 100 patients/group. This sample size gave 80% power to detect a 20% absolute difference in TIMI grade 2 or 3 recanalization at 60 min after treatment.

The primary objective of the trial was addressed using the 95% confidence interval (CI) for the difference in recanalization at 60 min after treatment. As a secondary objective, recanalization at 15, 30 and 45 min after treatment was also studied using 95% CI. Difference of recanalization rate 60 min after treatment in the two treatment groups was analyzed using chi-square analysis with the Yates correction for continuity.

Results

Clinical characteristics. A total of 211 patients were enrolled, 104 of whom were randomly assigned to receive E6010 and 107 native t-PA. Seven patients in the E6010 group and five in the native t-PA group were excluded from the analysis because the patients were either found to meet the exclusion criteria or the study protocol was not observed (i.e., in the E6010 group, mitral stenosis associated with atrial fibrillation in one patient, incomplete obstruction of the coronary artery before drug administration in two, >6 h from onset of myocardial infarction to initiation of administration in one, failure to receive the nitrate agent before therapy in two and fibrinolytic agent administered within 60 min of completion of intravenous infusion in one patient; in the native t-PA group, incomplete obstruction of the coronary artery before drug administration in two patients, saphenous vein bypass graft in one, fibrinolytic agent administered within 60 min of completion of intravenous infusion in one and no coronary angiography 60 min after start of intravenous infusion in one). As a result, 97 patients in the E6010 group and 102 in the native t-PA group provided evaluable angiograms.

Table 1 shows the baseline patient characteristics, which were similar in the two treatment groups. There were no significant differences between the two groups for any characteristic. The mean length of time from symptom onset to treatment initiation was also similar in the two groups. The actual dose of E6010 administered was 13.7 ± 2.4 mg and that of native t-PA was 28.8 mg.

Recanalization rate. Figures 1 and 2 show recanalization rates on the basis of coronary angiograms performed at 15-min intervals from the start of test drug administration. TIMI flow grade readings were made by interpreting the cine films in blind manner with regards to the identity of the agent administered to the patients. Recanalization rates for TIMI flow grade 2 or 3 and grade 3 alone in the E6010 and native t-PA groups are shown in Figures 1 and 2, respectively. At 60 min, the recanalization rates for TIMI flow grade 2 or 3, the primary end point, and TIMI grade 3 were significantly higher in the E6010 group than in the native t-PA group ($p < 0.05$). The recanalization rate increased with time after treatment in both groups, but E6010 produced a significantly higher recanalization rate than native t-PA at each assessment time ($p < 0.01$), the secondary end point, particularly at 15 and 30 min. The rate of recanalization for TIMI flow grade 3 at 60 min after a single bolus injection of 0.22 mg/kg of E6010 was 69% in a previous dose-finding study (4). With the same regimen, the rate of TIMI flow grade 2 or 3 recanalization was 78% at 60 min in the same study (4), consistent with the 79% observed in the present study.

Table 1. Baseline Characteristics

	E6010 Group (n = 97)	Native t-PA Group (n = 102)	p Value
Age (yr)	62 ± 11	63 ± 10	NS
Male (%)	81	76	NS
Body weight (kg)	62 ± 11	61 ± 10	NS
Previous MI (%)	7	12	NS
Time from symptom onset to treatment initiation (h)	3.5 ± 1.2	3.7 ± 1.3	NS
Site of infarction (%)			
Anterior	44	47	NS
Inferior	49	44	NS
Lateral	7	8	NS
Infarct-related artery (%)			
RCA	43	42	NS
LAD	45	47	NS
LCx	11	11	NS

Data presented are mean value ± SD or percent of patients. LAD = left anterior descending coronary artery; LCx = left circumflex coronary artery; MI = myocardial infarction; RCA = right coronary artery; t-PA = tissue-type plasminogen activator.

The rates of TIMI flow grade 2 or 3 recanalization and TIMI grade 3 recanalization alone were significantly higher in the E6010 group than in the native t-PA group regardless of the infarct-related artery or the time from onset of symptoms to treatment initiation ($p < 0.05$ by Cochran-Mantel-Haenszel test).

Adjunct therapy. During the first 60 min after test drug administration, use of other thrombolytic drugs, anticoagulant agents (other than heparin) and antiplatelet agents was prohibited. Only a total of 5,000 U of heparin was permitted immediately before coronary angiography. Antithrombotic cotherapy with aspirin, ticlopidine and argatroban was given without changing the dose during the study at the discretion of the attending physician, but there were no significant differences between the two groups in number of patients receiving these drugs (Table 2). Antiarrhythmic agents, calcium antagonists, nitrates, analgesics or sedatives and antishock therapy could be used freely.

At 60 min after test drug administration, additional thrombolytic therapy was permitted when judged necessary. Percutaneous transluminal coronary angioplasty and other types of adjunct therapy were permitted at the discretion of the attending physician.

Bleeding complications. Bleeding complications, including minor hemorrhage, occurred in eight E6010-treated and six

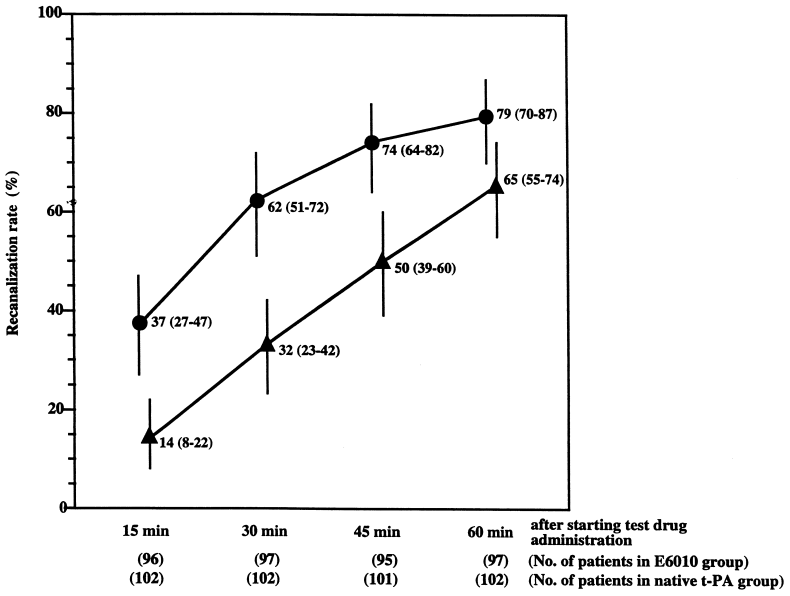

Figure 1. Recanalization rate of infarct-related arteries determined by coronary angiography at 15-min intervals. **Circles** = TIMI flow grade 2 or 3, E6010 group; **triangles** = TIMI flow grade 2 or 3, native t-PA group. 95% confidence intervals are shown in parentheses. $p < 0.01$, E6010 group versus native t-PA group at 15, 30 and 45 min. $p < 0.05$, E6010 group versus native t-PA group at 60 min.

native t-PA–treated patients (p = NS). Cerebral hemorrhage occurred in one patient who received native t-PA, resulting in death. One patient treated with E6010 experienced hematemesis requiring blood transfusion.

Changes in plasma fibrinolytic variables. Thirty-four patients in the E6010 and 42 in the native t-PA group who received no adjunct thrombolytic therapy were analyzed for the effects of test drug treatment on plasma fibrinolysis. Table 3 shows the changes in fibrinolytic variables over time. Decreases in plasminogen activity, alpha$_2$-plasmin inhibitor activity and fibrinogen levels were observed after both E6010 and native t-PA treatment. These variables returned to near-baseline levels 24 h after drug administration.

Clinical outcome. Death within 7 days of treatment occurred in four patients (4%) in the E6010 group and one (1%) in the native t-PA group (p = NS). Adjunct therapies included percutaneous transluminal coronary angioplasty and thrombolytic therapy, which were used in 54 patients in the E6010 group and 62 in the native t-PA group (Table 2). No patient underwent coronary artery bypass grafting. Among the patients receiving no adjunct therapy, reocclusion was seen in four (9%) in the E6010 group and six (15%) in the native t-PA group within 24 h and in five (13%) and seven (19%), respectively, within 7 days. Among the patients who demonstrated recanalization of TIMI flow grade 2 or 3 at 60 min after treatment, reinfarction developed in two (3%) in the E6010 group and four (6%) in the native t-PA group within 24 h, whereas four (5%) and seven (11%) patients, respectively, showed evidence of reinfarction within 7 days. However, these differences were not statistically significant.

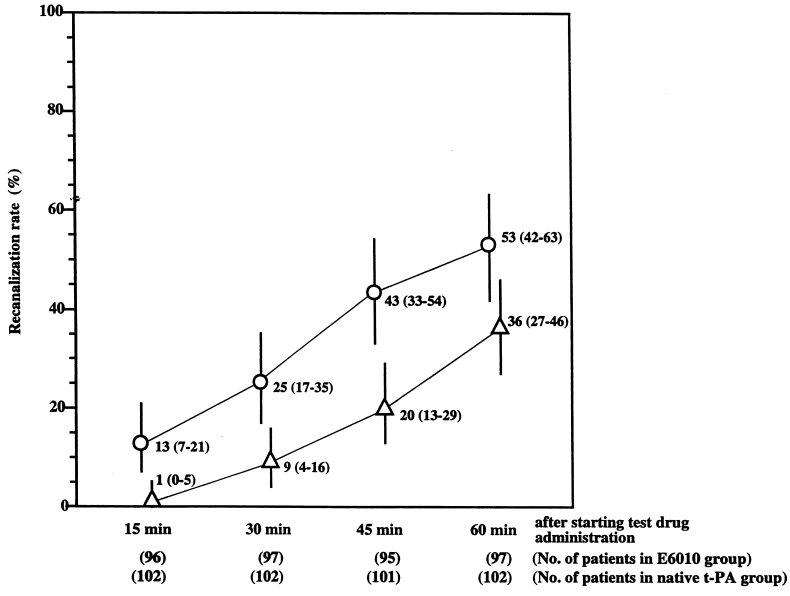

Figure 2. Recanalization rate of infarct-related arteries determined by coronary angiography at 15-min intervals. **Circles** = TIMI flow grade 3, E6010 group; **triangles** = TIMI flow grade 3, native t-PA group. 95% confidence intervals are shown in parentheses. $p < 0.01$, E6010 group versus native t-PA group at 15, 30 and 45 min. $p < 0.05$, E6010 group versus t-PA group at 60 min.

Table 2. Adjunctive Therapy

Group	Antithrombotic Cotherapy*	No Additional Reperfusion Therapy	Additional Reperfusion Therapy†
E6010 (n = 97)	73 (75.3%)	43 (44.3%)	54 (55.7%)
Native t-PA (n = 102)	85 (83.3%)	40 (39.2%)	62 (60.8%)

*Aspirin, ticlopidine or argatroban. †Percutaneous transluminal coronary angioplasty or thrombolytic agents, or both. Data presented are number (%) of patients. t-PA = tissue-type plasminogen activator.

Discussion

Thrombolytic treatment. A number of recent reports (10–12) have shown that early recanalization of the infarct-related coronary artery significantly improves cardiac function and reduces the mortality rate. Thus, rapid lysis of intracoronary thrombi with thrombolytic agents is a particularly important part of current treatment for acute myocardial infarction (1,13,14).

Tisokinase, the control drug in the present study, is one of two native t-PA preparations approved for clinical use in Japan. The tisokinase dose (28.8 mg infused over 60 min, with 2.8 mg of this dose given as a bolus) seems low and the evaluation of patency rate at 60 min unconventional compared with studies of native t-PA performed in the United States and Europe. However, this regimen of native t-PA (28.8 mg) (14.4 million IU, tisokinase) is currently a standard and extensively used method in Japan for the treatment of acute myocardial infarction, producing recanalization rates of 69% to 75% at 60 min, as reported in several Japanese studies (15,16). Plasminogen activating capacity is enhanced in the presence of fibrin. The percentage of tisokinase binding to fibrin was 68%, whereas that of alteplase was 66%; these values are practically equivalent. Therefore, it is thought that the plasminogen activating capacity of tisokinase is comparable to that of alteplase (17). The administered dose of tisokinase adjusted for body weight (assuming an average body weight of 60 kg for Japanese patients) was 0.48 mg/kg, whereas that of alteplase used in Japan is 0.5 mg/kg. In this regard, it is believed that the dose equivalences of tisokinase and alteplase were ensured in this study.

Comparison of the recanalization rate with higher doses, including higher initial doses, of native t-PA and E6010 is outside the scope of the present study. However, we would not expect much increase in the recanalization rate at 60 min with higher drug doses, without major bleeding complications in the Japanese population, given the recanalization rates at 60 min achieved in the present study (~80% and 70% with E6010 and native t-PA, respectively). The continuous intravenous infusion required for native t-PA is cumbersome and disadvantageous.

Pharmacokinetic considerations. The novel thrombolytic agent E6010 is a modified t-PA with a longer biologic half-life than that of native t-PA (2,3) and can thus be administered in patients with acute myocardial infarction as a single intravenous bolus injected over 2 min. Kinetic variables, area under the curve derived from analyses of E6010 and native t-PA

Table 3. Changes in Fibrinolytic Variables

Variable	Time of Measurement			
	Pre	60 min	120 min	24 h
Plasminogen activity (%)				
E6010 group	81.8 ± 17.6 (n = 34)	48.8 ± 14.5* (n = 34)	47.6 ± 13.1* (n = 34)	65.7 ± 14.2* (n = 30)
Native t-PA group	83.7 ± 19.9 (n = 42)	67.9 ± 19.9† (n = 41)	75.5 ± 12.9† (n = 40)	82.5 ± 15.9 (n = 40)
$Alpha_2PI$ activity (%)				
E6010 group	76.4 ± 19.9 (n = 32)	21.9 ± 14.6* (n = 32)	19.3 ± 14.4* (n = 32)	52.4 ± 19.9* (n = 27)
Native t-PA group	79.5 ± 19.9 (n = 40)	49.3 ± 21.7† (n = 39)	58.4 ± 16.8† (38)	68.9 ± 28.1‡ (38)
Fibrinogen level (mg/dl)				
E6010 group	268.7 ± 88.4 (n = 32)	182.7 ± 64.4* (n = 32)	170.2 ± 66.0* (n = 32)	200.9 ± 84.1§ (n = 27)
Native t-PA group	250.9 ± 95.2 (n = 42)	212.8 ± 83.5‖ (n = 41)	249.4 ± 73.3 (n = 39)	271.3 ± 101.3 (n = 40)
D-dimer level (ng/ml)				
E6010 group	227.8 ± 178.7 (n = 34)	832.1 ± 686.6* (n = 34)	1707.8 ± 1753.1* (n = 34)	950.1 ± 875.4* (n = 30)
Native t-PA group	210.7 ± 223.6 (n = 42)	582.7 ± 451.8† (n = 41)	949.6 ± 826.1† (n = 40)	672.3 ± 723.0† (n = 40)

*$p < 0.001$ and §$p < 0.01$ versus pretreatment (Pre) values for E6010 group. †$p < 0.001$, ‡$p < 0.01$ and ‖$p < 0.05$ versus pretreatment values for native tissue-type plasminogen activator (t-PA) by Wilcoxon signed rank test. Intergroup comparisons by the Student *t* test showed that decreases in all variables, except for the D-dimer level at 60 and 120 min and 24 h, were significantly greater in the E6010 group than in the native t-PA group ($p < 0.05$). Data presented are mean value ± SD. $Alpha_2PI$ = $alpha_2$-plasmin inhibitor.

antigens in plasma multiplied by the specific activity of each t-PA, representing total thrombolytic activity during the first 60 min after drug administration, obtained in Phase I studies in healthy volunteers (2), revealed that a single bolus injection of 13.2 mg (73,507 IU·min per ml) of E6010 (assuming the average body weight of Japanese patients to be 60 kg) was comparable to a continuous infusion of 28.8 mg (70,050 IU·min per ml) of native t-PA over 60 min (3). In a previous dose-finding study (4), E6010 administered as an intravenous bolus over 2 min in patients with an acute myocardial infarction was shown to have an optimal dose of 0.22 mg/kg on the basis of efficacy and safety.

Studies in an animal model of thrombosis have shown (18) that E6010 given as an intravenous bolus has excellent thrombolytic effects. In a canine model (19) in which coronary artery thrombi were induced using copper coils, intracoronary infusion of similar doses of E6010 and native t-PA showed that E6010 has more potent thrombolytic activity than native t-PA, as demonstrated by decreased time to reperfusion, increased reperfusion rate 60 min after treatment and decreased reocclusion rate 60 min after reperfusion (19). Thus, the superiority of E6010 is due to its formulation (i.e., a long plasma half-life that enables bolus administration), resulting in high effective plasma concentrations and, thus, a superior thrombolytic effect.

Recanalization rate. In the present study, coronary angiography was performed every 15 min to confirm whether early recanalization could be achieved. Although it is possible that the frequent infusion of contrast agent may have had some mechanical effect, the same procedures were performed in each group, providing valid comparative data. The percent of patients with TIMI flow grade 2 or 3 and flow grade 3 alone at each assessment and the recanalization rate 60 min after treatment were significantly better in patients treated with 0.22 mg/kg of E6010 as a 2-min bolus injection than that in patients treated with 28.8 mg (14.4 million IU) of native t-PA ($p < 0.05$). These findings indicate that E6010 produces an immediate improvement in coronary blood flow in the majority of patients. Recanalization at 60 min was used as the primary end point because the results of a preliminary dose-finding study (4) showed that TIMI flow grade 2 or 3 recanalization 60 min after drug administration was achieved in ~80% of patients using a single bolus of 0.22 mg/kg of E6010, and therefore there was no reason to expect an increase in the recanalization rate after an additional 30 min. Furthermore, it would have been unethical to wait an additional 30 min before starting adjunctive therapy that might have been beneficial to those patients in whom E6010 had not achieved coronary artery patency at 60 min. Moreover, the total thrombolytic activities of E6010 and native t-PA, represented by the kinetic variables, were maintained at similar levels for the 60 min after drug administration was initiated. Plasma native t-PA levels decrease rapidly immediately after discontinuation of administration. In the GUSTO Angiographic Study (1), patency was also evaluated at the completion of drug infusion.

Reteplase, which is a modified t-PA like E6010, has also achieved good patency rates: 77.6% and 85.2% at 60 and 90 min after administration, respectively (20). The patency rate at 60 min after administration (77.6%) is comparable to that of E6010 (79.0%). However, the plasma half-life of reteplase is shorter than that of E6010, and reteplase therefore requires double-bolus administration, with administration of a second bolus 30 min after the first bolus, whereas E6010 requires only single-bolus administration. E6010 is therefore advantageous in terms of convenience.

Bleeding complications. Bolus E6010 administration did not cause the occurrence of bleeding complications at significantly higher rates than those in the native t-PA group.

Fibrinolytic variables. The decreases in plasminogen activity, alpha$_2$-plasmin inhibitor activity and fibrinogen levels at 60 and 120 min and 24 h after treatment initiation were significantly greater in the E6010 group than in the native t-PA group ($p < 0.05$), probably because 1) giving E6010 as a bolus over 2 min causes the plasma drug level to increase soon after administration, and 2) E6010 has a lower affinity for fibrin than native t-PA. We suggest that a state of increased thrombolysis was maintained for a longer time in the E6010 group than in the native t-PA group because of the longer biologic half-life of E6010.

Reocclusion. The study protocol did not specify the adjunct therapy to be used. Therefore it would not be appropriate to compare the reocclusion rates in the E6010 and native t-PA groups. However, the reocclusion rate in the E6010 group within 7 days of treatment was similar to that observed for native t-PA in previous studies (21).

To prevent early reocclusion after thrombolytic therapy, heparin infusion and antiplatelet agents (including aspirin) were used. Because some patients who experienced reocclusion in this study were given these agents, the protective effect against reocclusion of such drugs may not necessarily be satisfactory. However, effective prevention of reocclusion using native t-PA combined with urokinase or streptokinase has been reported (22,23). Further study of the effect of adjunct therapy on reocclusion prevention is needed.

Conclusions. From the results presented here, we conclude that E6010, which can be administered as a single intravenous bolus injection over 2 min, produces a higher rate of early recanalization of infarct-related coronary arteries at 60 min than native t-PA, which is administered as a continuous intravenous infusion with an initial bolus injection of 10% of the total dose. This finding is important because it is crucial in clinical practice to achieve coronary artery patency within the "golden hours" in the treatment of acute myocardial infarction.

Appendix

Participating Investigators and Institutions for the E6010 Study Group

M. Nobuyoshi, Kokura Memorial Hospital; S. Suzuki, Sakakibara Heart Institute; H. Sato, Hiroshima City Hospital; M. Sugiyama, Yokohama City

University Urahune Hospital; T. Sone, Ogaki Municipal Hospital; F. Takatsu, Anjo Kosei Hospital; Y. Hiasa, Komatushima Red Cross Hospital; M. Kijima, Hoshi General Hospital; T. Motomiya, Tokyo Metropolitan Hiroo General Hospital; N. Nakamura, Matsue Red Cross Hospital; K. Hayasaki, Saiseikai Kumamoto Hospital; H. Abe, Hokko Cardiovascular Hospital; K. Kadowaki, Akita Medical Center; H. Murayama, Matsudo City Hospital; T. Joh, Ehime Prefectural Central Hospital; K. Kanmatsuse, Nihon University Surugadai Hospital; T. Katagiri, Showa University School of Medicine; S. Watanabe, Gifu Prefectural Hospital; K. Dohi, Nara Medical University; K. Kodama, Osaka Police Hospital; K. Tateda, Asahikawa City Hospital; M. Murata, Mito Saisei-Kai General Hospital; N. Hayashida, St. Luke International Hospital; Y. Yabe, School of Medicine, Toho University; M. Arita, Yokohama Minami Kyosai Hospital; K. Sakai, Tousei General Hospital; T. Kita, Cardiovascular Center, Sakakibara Hospital; H. Toda, Kagoshima Municipal Hospital; T. Meguro, Sendai Shakaihoken Hospital; M. Harada, Harada Heart Institute; N. Higuma, Niigata City General Hospital; T. Nagai, Nagaoka Red Cross Hospital; A. Takizawa, Shizuoka City Hospital; H. Hirayama, Nagoya Daini Red Cross Hospital; E. Murakami, Kanazawa University School of Medicine; K. Hirose, Ohtsu Red Cross Hospital; S. Ishise, Maizuru Kyosai Hospital; K. Shimomura, National Cardiovascular Center; M. Inada, Kansai Medical University; M. Chino, Ashikaga Red Cross Hospital; K. Hiramori, Iwate Medical University School of Medicine; K. Yokoyama, Yamagata Prefectural Central Hospital; T. Munakata, Sendai City Medical Center, Sendai Open Hospital; S. Takase, Saiseikai Maebashi Hospital; S. Saito, Nihon University Itabashi Hospital; Y. Dohi, Saitama Medical School; H. Nakamura, School of Medicine, National Defense Medical College; H. Nishimura, Kameda General Hospital; T. Takahashi, Komoro Kosei Hospital; H. Kanaya, Ishikawa Prefectural Hospital; M. Takahashi, Rakuyou Hospital; T. Minamino, Sakurabashi Watanabe Hospital; M. Yokoyama, Kobe University School of Medicine; T. Iwasaki, Hyogo College of Medicine; K. Mitsuto, Kurashiki Central Hospital; Y. Hayashi, Akanekai Tsuchiya General Hospital; T. Okuhara, Asa Municipal Hospital; M. Matsuzaki, Yamaguchi University School of Medicine; T. Yorozu, Yamaguchi Saiseikai General Hospital; H. Toshima, Kurume University School of Medicine; K. Ishikawa, Kyorin University School of Medicine; H. Ogawa, Tokuyama Chuoh Hospital; T. Syougase, Nikko Memorial Hospital; Y. Miyazawa, Cardiovascular Center of Sendai; H. Takeda, Semine Prefectural Hospital; A. Sibata, Niigata University School of Medicine; H. Yamaguchi, Juntendo University School of Medicine; S. Takahashi, Kosei General Hospital; M. Saito, Jichi Medical School, Omiya Medical Center; M. Nomura, Fujita Health University School of Medicine; J. Tsunekawa, Okazaki Municipal Hospital; H. Nishikawa, Yamada Red Cross Hospital; A. Wakabayashi, Kitano Hospital; J. Yoshikawa, Kobe General Hospital; K. Shoji, Okayama Red Cross Hospital; T. Sawayama, Kawasaki Medical School; E. Hyakuna, Shimonoseki Saikeikai General Hospital; H. Takeda, Kagawa Prefectural Central Hospital; Y. Kikuchi, Kyusyu Kose-Inenkin Hospital; T. Doi, Fukuoka Saiseikai General Hospital; S. Yano, Almeida Hospital; and H. Tanaka, Faculty of Medicine, Kagoshima University.

Principal Investigator: C. Kawai. **Steering Committee**: C. Kawai (*Chairman*), Y. Yui, S. Hosoda, M. Nobuyoshi, S. Suzuki, H. Sato, F. Takatsu, T. Motomiya, K. Kanmatsuse, K. Kodama, Y. Yabe, T. Minamino, and S. Kimata. **Controller**: M. Nakashima.

References

1. The GUSTO Angiographic Investigators. The effects of tissue plasminogen activator, streptokinase, or both on coronary-artery patency, ventricular function, and survival after acute myocardial infarction. N Engl J Med 1993;329:1615–22.
2. Ohnishi A, Takazawa K, Fujita M, et al. Phase I study of the modified tissue plasminogen activator E6010 [in Japanese]. Jpn J Clin Pharmacol Ther 1994;25:551–62.
3. Mori T, Nishino N, Shizume K, et al. Change in various parameters of fibrinolysis in persons infused with tissue plasminogen activator: special reference to plasminogen activator inhibitor [in Japanese]. Jpn Pharmacol Ther 1988;16:1589–96.
4. Kawai C, Hosoda S, Motomiya T, et al., and the E6010 investigators. Multicenter trial of a novel modified t-PA, E6010, by bolus injection in patients with acute myocardial infarction [abstract]. Circulation 1992;86 Suppl I:I–409.
5. Chesebro JH, Knatterud G, Roberts R, et al. Thrombolysis in Myocardial Infarction (TIMI) trial, Phase I: a comparison between intravenous tissue plasminogen activator and intravenous streptokinase. Circulation 1987;76: 142–54.
6. Mohler MA, Refino CJ, Chen SA, Chen AB, Hotchkiss AJ. D-Phe-Pro-Arg-chloromethylketone: its potential use in inhibiting the formation of in vitro artifacts in blood collected during tissue-type plasminogen activator thrombolytic therapy. Thromb Haemost 1986;56:160–4.
7. Friberger P, Knös M, Gustavsson S, Aurell L, Claeson G. Methods of determination of plasmin, antiplasmin and plasminogen by means of substrate S-2251. Haemostasis 1978;7:138–45.
8. Dati F, Barthels M, Conard J, et al. Multicenter evaluation of a chromogenic substrate method for photometric determination of prothrombin time. Thromb Haemost 1987;58:856–65.
9. Elms MJ, Bunce IH, Bundesen PG, et al. Measurement of crosslinked fibrin degradation products: an immunoassay using monoclonal antibodies. Thromb Haemost 1983;50:591–4.
10. Gruppo Italiano per lo Studio della Streptochinasi nell'Infarto Miocardico (GISSI). Effectiveness of intravenous thrombolytic treatment in acute myocardial infarction. Lancet 1986;1:397–402.
11. Simoons ML, Serruys PW, van den Brand M, et al., and Working Group on Thrombolytic Therapy in Acute Myocardial Infarction of the Netherlands Interuniversity Cardiology Institute. Early thrombolysis in acute myocardial infarction: limitation of infarct size and improved survival. J Am Coll Cardiol 1986;7:717–28.
12. Van de Werf F, Arnold AER. Intravenous tissue plasminogen activator and size of infarct, left ventricular function, and survival in acute myocardial infarction. BMJ 1988;297:1374–9.
13. Carney RJ, Murphy GA, Brandt TR, et al., and the RAAMI Study Investigators. Randomized angiographic trial of recombinant tissue-type plasminogen activator (alteplase) in myocardial infarction. J Am Coll Cardiol 1992;20:17–23.
14. The GUSTO Investigators. An international randomized trial comparing four thrombolytic strategies for acute myocardial infarction. N Engl J Med 1993;329:673–82.
15. Hirosawa K, Suzuki S, Kawai C, Yui Y. Intravenous coronary thrombolysis in acute myocardial infarction by AK-124 (tissue plasminogen activator): multicenter, double-blind study in comparison with urokinase [in Japanese]. Jpn Pharmacol Ther 1991;19:1003–32.
16. Kanemoto K, Goto Y, Hirosawa K, Kawai C. Intravenous recombinant tissue-type plasminogen activator in patients with acute myocardial infarction—a report from the multicenter thrombolysis trial. Jpn Circ J 1990;54: 71–81.
17. Murakami A, Yoshizaki H, Shirato S, Kondou S, Kiyota T, Hayashi H. Characterization of tissue plasminogen activator (AK-124) in fibrinolysis [in Japanese]. Jpn Pharmacol Ther 1991;19:59–64.
18. Suzuki S, Saito M, Suzuki N, et al. Thrombolytic properties of a novel modified human tissue-type plasminogen activator (E6010): a bolus injection of E6010 has equivalent potency of lysing young and aged canine coronary thrombi. J Cardiovasc Pharmacol 1991;17:738–46.
19. Suzuki S, Saito M, Suzuki N, et al. Intracoronary infusion of E6010 has more potent thrombolytic activity than tissue plasminogen activator (t-PA) in dogs: a higher plasma level of E6010 than t-PA causes potent thrombolytic activity. J Cardiovasc Pharmacol 1993;22:834–40.
20. Smalling RW, Bode C, Kallfleisch J, et al., and the RAPID Investigators. More rapid, complete, and stable coronary thrombolysis with bolus administration of reteplase compared with alteplase infusion in acute myocardial infarction. Circulation 1995;91:2725–32.
21. Califf RM, Topol EJ, Stack RS, et al., and the TAMI Study Group. Evaluation of combination thrombolytic therapy and timing of cardiac catheterization in acute myocardial infarction: results of Thrombolysis and Angioplasty in Myocardial Infarction—Phase 5 randomized trial. Circulation 1991;83:1543–56.
22. Topol EJ, Califf RM, George BS, et al., and the TAMI Study Group. Coronary arterial thrombolysis with combined infusion of recombinant tissue-type plasminogen activator and urokinase in patients with acute myocardial infarction. Circulation 1988;77:1100–7.
23. Grines CL, Nissen SE, Booth DC, et al., and the KAMIT Study Group. A prospective, randomized trial comparing combination half-dose tissue-type plasminogen activator and streptokinase with full-dose tissue-type plasminogen activator. Circulation 1991;84:540–9.